

WHAT IS CLAIMED IS:

1. A molecular fingerprinting method comprising the steps of:
  - (a) identifying a target polynucleotide,
  - (b) selecting at least one fragment of the target polynucleotide, wherein the fragment is a fixed distance from a restriction site, to generate a set of one or more polynucleotide fragments, and
  - (c) designating some or all of the set of fragments as molecular fingerprint corresponding to the target polynucleotide.
2. A method of claim 1 wherein each fragment is selected at random.
3. A method of claim 2 wherein each fragment is about 20 to about 50 base pairs in length.
4. A method for identifying a polynucleotide sample comprising the steps of:
  - (a) identifying a target polynucleotide;
  - (b) selecting at least one fragment of the target polynucleotide, wherein the fragment is a fixed distance from a restriction site, to generate a set of one or more polynucleotide fragments;
  - (c) designating some or all of the set of fragments as a fingerprint corresponding to the target polynucleotide;
  - (d) synthesizing one or more oligonucleotide probes to complement the set of polynucleotide fragments;
  - (e) combining the probes, a polynucleotide sample, nucleotide triphosphates, and polymerase to synthesize at least one polynucleotide strand;
  - (f) cutting the strands with restriction enzymes to yield a set of sample fragments of fixed length; and

(g) comparing the set of sample fragments to the fingerprint.

5. A method of claim 4, wherein the polynucleotide sample is digested before the combining step.

6. A method of claim 5, wherein digestion is performed with a digestion enzyme.

7. A method of claim 6, wherein the digestion enzyme is a six-base cutter.

8. A method of claim 5, wherein the polynucleotide sample is digested into fragments of tens of thousands of base pairs.

9. A method of claim 4 wherein the nucleotide triphosphates are fluorescently labeled.

10. A method of claim 4, wherein the sample fragments are compared to the fingerprint by determining the sizes of the sample fragments in relation to the fingerprint fragments.

11. A method according to claim 10 wherein determining the sizes of fragments comprises gel electrophoresis.

12. A method according to claim 10 wherein determining the sizes of fragments comprises discrimination in a microfluidic device.

13. A method of claim 4, wherein the polynucleotide sample is digested after the combining step.

14. A method according to claim 4, wherein the sample polynucleotide is a forensic sample.

15. A method according to claim 4, wherein the target polynucleotide is associated with a disease.

16. A method for detecting a particular nucleic acid in a sample, which particular nucleic acid has at least one restriction site, and which method comprises:

- (a) contacting the sample with
  - a primer that hybridizes to the particular nucleic acid a predetermined distance from the restriction site,
  - a polymerase and
  - a plurality of nucleotides,so that a complementary nucleic acid is synthesized from the primer at least to the restriction site;
- (b) contacting the complementary nucleic acid with a restriction enzyme under conditions capable of cutting the complementary nucleic acid at the restriction site; and
- (c) detecting a nucleic acid fragment having a particular length equal to the fixed distance,

wherein the presence of the nucleic acid fragment in the sample indicates that the particular nucleic acid is present in the sample.

17. A method according to claim 16 wherein the primer comprises an oligonucleotide about 20-50 nucleotides in length.

18. A method according to claim 16 wherein at least some of the plurality of nucleotides are detectably labeled.

19. A method according to claim 18 wherein the labeled nucleotides are fluorescently labeled.

20. A method according to claim 16 in which no more than a single complementary nucleic acid molecule is synthesized.

21. A method according to claim 16 in which the nucleic acid fragment is detected using a device for processing a flow of polynucleotide molecules, which device comprises a substrate and an analysis unit, wherein the analysis unit comprises:

a main channel having a polynucleotide sample inlet, a detection region downstream of the sample inlet and an outlet region adjacent to and downstream of the detection region; and

a detector sensitive to polynucleotides passing through the detector region, and wherein, on average, one polynucleotide at a time is placed within the detection region.

22. A method according to claim 21 wherein the channels of the device are about 1-100  $\mu\text{m}$  in depth.

23. A method according to claim 21 wherein the detection region of the device has a volume between about 1 femtoliter and 1 nanoliter.

24. A method according to claim 21 wherein the detector is sensitive to the size of polynucleotide molecules passing through the detection region.

25. A method according to claim 21 wherein:  
at least some of the plurality of nucleotides are detectably labeled; and

the detector is sensitive to the detectable label.

26. A method according to claim 25 wherein the labeled nucleotides are fluorescently labeled.

27. A method according to claim 16 wherein the nucleic acid fragment is detected according to a method that comprises:

- (i) sorting polynucleotide molecules in the sample according to size; and
- (ii) identifying a polynucleotide having the particular length.

28. A method according to claim 27 in which polynucleotide molecules are sorted by gel electrophoresis.

29. A method according to claim 27 in which polynucleotide molecules are sorted by HPLC.

30. A method according to claim 27 in which polynucleotide molecules are sorted in a microfluidic device.

31. A method according to claim 27 in which polynucleotide molecules are sorted in a device for processing a flow of polynucleotide molecules, which device comprises a substrate and an analysis unit, wherein the analysis unit comprises:

a main channel having a polynucleotide sample inlet, a detection region downstream of the sample inlet and a branch point discrimination region adjacent to and downstream of the detection region, wherein on average one polynucleotide molecule at a time is placed within the detection region;

at least two branch channels originating at the branch point discrimination region  
and in communication with the main channel;  
a detector sensitive to polynucleotide molecules passing through the detection  
region; and  
a flow control responsive to the detector and acting to direct polynucleotide  
molecules at the discrimination region into a selected branch channel.

32. A method according to claim 31 wherein the channels of the device are  
about 1-100  $\mu\text{m}$  in depth.

33. A method according to claim 31 wherein the detection region of the device  
has a volume between about 1 femtoliter and 1 nanoliter.

34. A method according to claim 31 wherein:  
at least some of the plurality of nucleotides are detectably labeled, and  
polynucleotides are directed to a selected branch channel based on a  
measured level of the detectable label.

35. A method according to claim 34 wherein the labeled nucleotides are  
fluorescently labeled.

36. A method for detecting a particular nucleic acid in a sample,  
which particular nucleic acid has at least one restriction site, and  
which method comprises:

- (a) contacting the sample with a restriction enzyme under conditions capable  
of cutting the particular nucleic acid at the restriction site;
- (b) contacting the sample with  
a primer that hybridizes to the nucleic acid a predetermined distance from  
the cut at the restriction site,

a polymerase and  
a plurality of nucleotides,  
so that a complementary nucleic acid fragment is synthesized; and  
(c) detecting the complementary to nucleic acid fragment,  
wherein the presence of the complementary nucleic acid fragment in the sample indicates  
that the particular nucleic acid is present in the sample.

37. A method according to claim 36 wherein the primer comprises an  
oligonucleotide about 20-50 nucleotides in length.

38. A method according to claim 36 wherein at least some of the plurality of  
nucleotides are detectably labeled.

39. A method according to claim 38 wherein the labeled nucleotides are  
fluorescently labeled.

40. A method according to claim 36 in which no more than a single  
complementary nucleic acid fragment is synthesized.

41. A method according to claim 36 in which the nucleic acid fragment is  
detected using a device for processing a flow of polynucleotide molecules,  
which device comprises a substrate and an analysis unit,  
wherein the analysis unit comprises:

a main channel having a polynucleotide sample inlet, a detection region  
downstream of the sample inlet and an outlet region adjacent to and  
downstream of the detection region; and

a detector sensitive to polynucleotides passing through the detector region,  
and wherein, on average, one polynucleotide at a time is placed within the detection  
region.

42. A method according to claim 41 wherein the channels of the device are about 1-100  $\mu\text{m}$  in depth.

43. A method according to claim 41 wherein the detection region of the device has a volume between about 1 femtoliter and 1 nanoliter.

44. A method according to claim 41 wherein the detector is sensitive to the size of polynucleotide molecules passing through the detection region.

45. A method according to claim 41 wherein:  
at least some of the plurality of nucleotides are detectably labeled; and  
the detector is sensitive to the detectable label.

46. A method according to claim 45 wherein the labeled nucleotides are fluorescently labeled.

47. A method according to claim 36 wherein the nucleic acid fragment is detected according to a method that comprises:

- (i) sorting polynucleotide molecules in the sample according to size; and
- (ii) identifying a polynucleotide having the particular length.

48. A method according to claim 47 in which polynucleotide molecules are sorted by gel electrophoresis.

49. A method according to claim 47 in which polynucleotide molecules are sorted by HPLC.

50. A method according to claim 47 in which polynucleotide molecules are sorted in a microfluidic device.



51. A method according to claim 47 in which polynucleotide molecules are sorted in a device for processing a flow of polynucleotide molecules, which device comprises a substrate and an analysis unit, wherein the analysis unit comprises:

- a main channel having a polynucleotide sample inlet, a detection region downstream of the sample inlet and a branch point discrimination region adjacent to and downstream of the detection region, wherein on average one polynucleotide molecule at a time is placed within the detection region;
- at least two branch channels originating at the branch point discrimination region and in communication with the main channel;
- a detector sensitive to polynucleotide molecules passing through the detection region; and
- a flow control responsive to the detector and acting to direct polynucleotide molecules at the discrimination region into a selected branch channel.

52. A method according to claim 51 wherein the channels of the device are about 1-100  $\mu\text{m}$  in depth.

53. A method according to claim 51 wherein the detection region of the device has a volume between about 1 femtoliter and 1 nanoliter.

- 54. A method according to claim 51 wherein:
  - at least some of the plurality of nucleotides are detectably labeled, and
  - polynucleotides are directed to a selected branch channel based on a measured level of the detectable label.

55. A method according to claim 51 wherein the labeled nucleotides are fluorescently labeled.

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